

Attorney Docket No.: RU-0191  
Inventors: Allan H. Conney  
Serial No.: 10/657,685  
Filing Date: September 8, 2003  
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**Amendments to the Specification:**

Please remove the paragraph beginning on page 4, line 23:

~~Figure 7 depicts the number of viable cells present after paclitaxol (4 or 10 ng/ml) treatment of LNCaP cells seeded at a density of  $1 \times 10^5$  cells/ml and incubated for 48 hours with either TPA (1 ng/ml) or vehicle.~~

Please replace the paragraph beginning on page 13, line 8, with the following rewritten paragraph:

Studies were also performed to examine the potential synergistic activity of TPA combined in therapy with paclitaxol. Paclitaxol is a well-known chemotherapeutic agent sold under the trade name of Taxol® and currently approved by the U.S. Food and Drug Administration for the treatment of breast cancer, ovarian cancer, small cell lung carcinoma, and AIDS-related Kaposi's sarcoma. Male NCr immunodeficient mice were injected subcutaneously with LNCaP cells in matragel. After 4 to 6 weeks, mice with tumors (0.65-1 cm long and 0.65-1 cm wide) were randomly assigned to 4 groups (6 mice per group). Animals in group 1 received i.p. injections of vehicle (5  $\mu$ /g body weight), animals in group 2 received i.p. injections of TPA (100 ng/g; 5  $\mu$ l vehicle/g), animals in group 3 received i.p. injections of

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paclitaxol (10 ng/g; 5  $\mu$ l vehicle/g), and animals in group 4 received i.p. injections of TPA (100 ng/g) in combination with paclitaxol (10 ng/g; 5  $\mu$ l vehicle/g), once a day for 5 days followed by a 2 day intermission. The mice received treatment for 28 days. The vehicle consisted of polypropylene glycol, polysorbate 80, benzyl alcohol, ethanol and water (40:0.5:1:10:48.5, respectively). Tumor size was measured and expressed as percent of initial size. The results of these experiments are shown in Figure 6. Combined treatment of TPA and paclitaxol (Taxol®) resulted in a synergistic effect. Tumor size was significantly smaller in animals treated with the combined therapy. Although treatment of LNCaP cells with TPA or paclitaxol alone for 48 or 96 hours had only small effects on apoptosis, treatment of these cells with a combination of TPA and paclitaxol, especially for 96 hours, resulted in a marked stimulation of apoptosis as measured by the percent of pre-G<sub>0</sub>/G<sub>1</sub> cells using flow cytometry. These results are shown below in Table 4.

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| Table 4   |  |                         |                 |                       |                         |
|---|--|-------------------------|-----------------|-----------------------|-------------------------|
| Effects of TPA or Taxol Alone or in Combination on<br>Proliferation, Cell Cycle Distribution and Apoptosis in<br>Cultured LNCaP Cells |  |                         |                 |                       |                         |
| Treatment   | # Viable<br>Cells<br>( $1 \times 10^5$ ) | % Cells<br>in $G_0/G_1$ | % Cells<br>in S | % Cells<br>in $G_2/M$ | %<br>Apoptotic<br>Cells |
| Treatment for 48 Hours  |  |                         |                 |                       |                         |
| Untreated<br>control  | 8.9                                      | 76.5                    | 16.1            | 5.2                   | 1.6                     |
| Ethanol   | 0.0                                      | 76.8                    | 16.4            | 4.6                   | 1.8                     |
| TPA<br>1 ng/ml  | 3.7                                      | 77.8                    | 13.0            | 5.5                   | 10.1                    |
| Taxol<br>5 ng/ml  | 4.6                                      | 73.0                    | 12.8            | 6.4                   | 12.9                    |
| TPA +<br>Taxol<br>(doses as<br>above)   | 1.3                                      | 58.6                    | 12.6            | 5.3                   | 28.3                    |
| Treatment for 96 Hours  |  |                         |                 |                       |                         |
| Untreated<br>control  | 10.6                                     | 85.7                    | 7.5             | 5.2                   | 1.2                     |
| Ethanol   | 12.3                                     | 83.6                    | 7.9             | 6.2                   | 1.9                     |
| TPA<br>1 ng/ml  | 3.2                                      | 66.7                    | 12.3            | 9.5                   | 11.2                    |

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|                                       |     |      |      |     |      |
|---------------------------------------|-----|------|------|-----|------|
| Taxol<br>5 ng/ml                      | 2.5 | 49.7 | 12.4 | 8.7 | 28.4 |
| TPA +<br>Taxol<br>(doses as<br>above) | 0.4 | 29.9 | 10.1 | 8.0 | 50.8 |

Experiments were also performed to determine the number of viable cells present after paclitaxol (4 or 10 ng/ml) treatment of LNCaP cells seeded at a density of  $1 \times 10^5$  cells/ml and incubated for 48 hours with either TPA (1 ng/ml) or vehicle. Treatment of cells in culture with paclitaxol in combination with TPA resulted in a synergistic effect on cell viability (~~see Figure 7~~). Therefore, a combination of TPA and paclitaxol also was shown to be an effective treatment for inhibition of prostate tumor cell growth.

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**Amendment to the Drawing Figures:**

Please remove Figure 7.